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(54) Title: SILOXY OLIGONUCLEOTIDE ANALOGS

(57) Abstract

A class of oligodeoxyribonucleotide and oligoribonucleotide analogs containing one or more stable internucleotide siloxy linkages is presented here. The linkages, in which the phosphodiester group is replaced by a siloxy group, are neutral, provide achiral centers, and are completely nuclease resistant. The siloxy-modified oligonucleotides are easy to synthesize, flexible enough to allow for the production of a diverse family of compounds, and exhibit nucleic acid hybridization properties essentially identical to those of unmodified oligonucleotides. This class of oligonucleotide is ideal for use in therapeutic administrations and diagnostic applications.

SILOXY OLIGONUCLEOTIDE ANALOGS

1. INTRODUCTION

5   A class of oligodeoxyribonucleotide and
oligoribonucleotide analogs (referred to herein as
"oligonucleotide analogs") containing one or more
stable internucleotide siloxy linkages is presented
here. Such oligonucleotides may be single or double
10   stranded. These linkages, in which the phosphodiester
group is replaced by a siloxy group, are neutral,
provide achiral centers, and are completely nuclease
resistant. Synthesis of these siloxy-modified
oligonucleotides is very easy, requiring no specially
15   modified nucleosides, and is flexible enough to allow
for the production of a diverse family of compounds.
In addition, the hybridization properties of
oligonucleotides containing siloxy linkages are
essentially identical to unmodified oligonucleotides
20   containing only phosphodiester linkages. Thus, this
easily produced oligonucleotide class is ideal for use
in therapeutic administration of oligonucleotides that
can be targeted toward the treatment of a large number
of deleterious processes and disorders, ranging from
25   viral infection to malignant growth. In addition to
their therapeutic uses, the siloxy oligonucleotide
analogs may be used in any case where the expression
of a specific gene is to be modulated, and may
alternatively be used in diagnostic procedures where
30   resistance to nucleases, such as is the case with in
situ hybridizations, is required.

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- 2 -

2.   BACKGROUND OF THE INVENTION

2.1   THERAPEUTIC AND DIAGNOSTIC USES OF
      OLIGONUCLEOTIDES

The ability to control the expression of harmful
genetic information is a fundamental goal of medical,
molecular, and biochemical sciences.   One potential
method for accomplishing such control was first
demonstrated by Zamecnik and Stephenson, (Zamecnik,
P.C. and Stephenson, M.L., 1978, Proc. Natl. Acad.
Sci. USA 75:280-284) who showed that the introduction
of synthetic oligonucleotides into growing cells could
interfere with the cell's susceptibility to viral
infection, and later by Melton (Melton, D.A., 1985,
Proc. Natl. Acad. Sci. USA 82:144-148) who
demonstrated that oligonucleotides could down-regulate
the expression of specific cellular genes.   Since
then, due to the great potential this technique holds
for the treatment of a wide range of disorders and
deleterious processes, the design of strategies and
methods to modulate the expression of cellular or
viral genes through the introduction of exogenous
oligonucleotides has been a focus of research.   By
selectively blocking the expression of particular
genes, oligonucleotides may be used therapeutically,
for example, to specifically inhibit viral or
bacterial infections, or to slow the proliferation of
cancerous cells.   For recent reviews, see Cohen, J.S.,
1991, Pharmacol. Ther. 52:211-215 and Crooke, S.T.,
1992, Ann. Rev. Pharmacol. Toxicol. 32:329-376.

Oligonucleotide inhibition of gene expression may
be post-transcriptional.   Here, oligodeoxyribo-
nucleotides (referred to with respect to this process
as "antisense oligonucleotides") having a nucleotide
sequence complementary to a portion of a specified

mRNA bind to that mRNA, causing the expression of the corresponding gene to be blocked. It has been shown that the predominant mechanism for such expression inhibition is the degradation of the mRNA in such an

5 RNA/DNA heteroduplex by the enzyme RNase H (Walder, R.Y. and Walder, J.A., 1988, Proc. Natl. Acad. Sci. USA 85:5011-5015). The use of oligodeoxyribonucleotides in conjunction with RNase H has the potential advantage of acting as a catalytic process.

10 This is due to the fact that many copies of a particular transcript may be degraded for every oligodeoxyribonucleotide introduced, since RNase H only cleaves the RNA, not the DNA in a heteroduplex, meaning that once an mRNA is degraded, the

15 oligodeoxyribonucleotide is freed and can hybridize to another transcript. Additionally, oligonucleotides can function to post-transcriptionally inhibit gene expression in an RNase H-independent manner by physically blocking the ribosome from productively

20 interacting with the RNA (Yu, Z. et al., 1989, J. Exper. Pathol.4:97-108). The level of inhibition via this mechanism appears to be dependent on the presence or absence of secondary structure at the binding site of the oligonucleotide (Blake, K.R. et at., 1985,

25 Biochemistry 24:6139-6145).

In addition to a post-transcriptional level of control of gene expression, oligonucleotide inhibition of gene expression may also occur at the transcriptional level (McShan, W.M. et al., 1992, J.

30 Biol. Chem. 267: 5712-5721; Postel, E.H. et al., 1991, Proc. Natl. Acad. Sci. USA 88:8227-8231). Here, an oligonucleotide can interfere with transcription of a specific mRNA, through the formation of a triple helix with the endogenous double stranded DNA via Hoogsteen,

35 as opposed to the usual Watson-Crick, base pairing. A

- 4 -

potential advantage to this approach is that only one
molecule of oligonucleotide is required for each copy
present of the gene to be inhibited. Another approach
to oligonucleotide-driven inhibition of gene
5   expression that also acts at the transcriptional level
has been used by Bielinska et al. (Bielinska, A. et
al., 1990, Science 250:997-1000). Here, the Bielinska
group employed double-stranded oligonucleotide analogs
to inhibit gene expression by using them to compete
10   inside the nucleus with endogenous promoter sequences
for the binding of specific transcription factors.
       The use of RNA oligonucleotides, termed
ribozymes, may also provide a means by which to
inhibit specific gene expression. This method takes
15   advantage of the fact that mRNA splicing occurs via
autocatalytic RNAs, which cleave RNA through the
enzymatic use of the 2'-OH of a specific sequence
(Cech, T.R., 1986, Cell 44:207-210). Ribozymes are
designed to hybridize to a specific sequence of RNA
20   and cleave this target RNA by transesterification. By
targeting a single mRNA species, gene expression may
be controlled in a specific manner. Catalytic RNA
cleavage by ribozymes occurs independent of any
protein. Once cleavage is completed, the ribozyme is
25   freed to bind to a new target RNA.
       Recently, a technique has been described that
could dramatically increase the potential of
oligonucleotide-based therapeutics (Blackwell, T.K.
and Weintraub, 1990, Science 250:1104-1110; Blackwell,
30   T.K. and Weintraub, 1990, Science 250:1149-1151;
Ellington, A.D. and Szostak, J., 1990, Nature 346:818-
822; Tuerk, C. and Gold, L., 1990, Science 249:505-
510). This procedure consists, first, of the
synthesis of large numbers of random-sequence
35   oligonucleotides. Pools of these oligonucleotides,

- 5 -

containing upwards of 10^{13} different sequences, are
produced. Such oligonucleotides may be DNA or RNA,
and either single or double stranded. Next, sequences
are selected that, by chance, have the correct three-
5   dimensional structure to bind a target molecule. The
target molecule may range from a small organic
molecule to a large protein. In this manner,
oligonucleotides (referred to with respect to this
process as "aptamers") can be selected that bind with
10   high affinity to any molecule whose inhibition may be
of therapeutic interest. For example, any
extracellular molecule may be targeted, circumventing
the need for cell permeation, described below, that is
faced with traditional applications of oligonucleotide
15   therapies.

In addition to therapeutics, the field of DNA
diagnostics is also under very active investigation,
having applications for the diagnosis of infectious
diseases, cancer, and genetic disorders. Here, a
20   nucleic acid probe, often an oligonucleotide, is used
to detect the presence of a complementary nucleic acid
(DNA or RNA sequence). In this process, the probe
hybridizes to its complementary sequence if it is
present within the sample. The target sequence may be
25   analyzed in solution or, as is frequently the case, it
may first be immobilized on a solid support, such as
nitrocellulose or a nylon membrane. The probe carries
a label, e.g., a radioactive, fluorescent, or enzyme
marker, to permit their detection. Existing methods,
30   although extremely useful as research tools, often
lack the sensitivity necessary for many practical
applications.

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- 6 -

2.2 FACTORS AFFECTING EFFICACY OF OLIGONUCLEOTIDES FOR THERAPEUTIC ADMINISTRATION AND DIAGNOSTIC APPLICATIONS

One consideration currently affecting the utility of oligonucleotides as potential therapeutic agents is the rapid enzymatic nuclease degradation that the oligonucleotides undergo in the bloodstream and within cells. Unmodified oligonucleotides are degraded sufficiently rapidly in blood, and even more quickly in cells, that their effect as drugs becomes abrogated. In addition, at least part of the suboptimal sensitivity observed in diagnostic applications may also be due to such nuclease degradation. Although efforts are made to remove contaminating nucleases from clinical samples to be analyzed, often low levels may remain. This can be a major problem in such techniques as _in situ_ hybridizations, where probes are hybridized directly to tissue samples.

Nucleases are enzymes that hydrolyze the phosphodiester bonds joining the nucleotides within a DNA or RNA chain, thereby causing degradation by cleaving the molecule into smaller and smaller fragments. Nucleases are classified into two categories, endonucleases, which can cleave phosphodiester bonds located at any point along a nucleic acid chain, and exonucleases, which can only cleave terminal phosphodiester bonds. Exonucleases are further divided into those that cleave from the 5' end of the nucleic acid molecule inward (5'→3'), and those that cleave from the 3' end of the nucleic acid molecule inward (3'→5'). It has been demonstrated that the predominant mechanism responsible for the

- 7 -

rapid degradation of oligonucleotides is a 3'→5'
mechanism (Walder, J.A. _et al._, 1989, WO 89/05358),
although there is a minor effect observed from 5'→3'
exonucleases as well.

In the past, progress has been made in the
development of oligonucleotide analogs that are
resistant to nuclease degradation. These
modifications have included the replacement of the
phosphodiester groups of the DNA backbone with either
phosphorothioate (Matsukura, M. _et al._, 1987, Proc.
Natl. Acad. Sci. USA 84:7706-7710) or phosphoramidate
(Agrawal, S. _et al._, 1988, Proc. Natl. Acad. Sci. USA
87:7079-7083; Bartlett, P.A. and Marlowe, C.K., 1983,
Biochemistry 22:4618) groups, and the production of
neutral DNA analogs using methyl phosphonates (Miller
P.S. _et al._, 1985, Biochimie 67:769-776; Smith, C.C.
_et al._, Proc. Natl. Acad. Sci. USA 83:2787-2791) or
phosphate triesters (Miller, P.S. _et al._, 1982,
Biochemistry 21:5468; Moody, H.M. _et al._, 1989,
Nucleic Acids Res. 17:4769). Alternatively, DNA
analogs have been produced in which the phosphate has
been replaced altogether by substituting it with
carbamates (Coull, J.M. _et al._, 1987, Tetrahedron
Lett. 28:745; Stirchak, E.P. and Summerton, J.E.,
1987, J. Org. Chem. 52:4202), formacetal groups
(Matteucci, M., 1990, Tetrahedron Lett. 31:2385-2388;
Matteucci, M., 1991, Nucleos. and Nucleot. 10:231-234;
Matteucci, M. _et al._, 1991, J. Am. Chem. Soc.
113:7768-7770), sulfur, via sulfide, sulfoxide,
sulfone or sulfonyl groups (Huang, Z., _et al._, 1991,
J. Org. Chem. 56:3869-3882; Musicki, B. and Widlanski,
J.S., 1991, Tetrahedron Lett. 32:1267-1270; Musicki,
B. and Widlanski, J.S., 1991, J. Organic Chem.
55:3230-3232; Schneider, K.C. and Benner, S.A., 1990,
Tetrahedron Lett. 31:335-338), or silyl (Si-O) groups

- 8 -

(Cormier, J.F. and Ogilvie, K.K., 1988, Nucleic Acids
Res. 16:4583-4594; Ogilvie, K.K. and Cormier, J.F.,
1985, Tetrahedron Lett. 26:4159-4162; Selgier H. and
Feger, G., 1987, Nucleos. and Nucleot. 6:483-484).
5   Finally, a radically different oligonucleotide analog
has been reported in which the entire phophodiester-
sugar backbone has been replaced by a polyamide chain
(Nielson, P. et al., 1991, Science 254:1497-1500).
        While each of the above mentioned classes of
10   oligonucleotide analogs exhibits some degree of
nuclease resistance, the use of such derivatives to
modulate the expression of specifically targeted genes
has met with limited success on account of one or more
other factors. Among these other factors of
15   importance in determining the usefulness of
oligonucleotides as potential therapeutic agents are
the oligonucleotides' affinity for DNA (for use in
triple helix formation) and RNA. The relative ability
of an oligonucleotide to bind to complementary nucleic
20   acids is compared by determining the melting
temperature of a particular hybridization complex.
The melting temperature (T_m) denotes the temperature
at which 50% of the double helices have dissociated
into single stranded molecules. The higher the T_m,
25   the greater the strength of the binding of the
strands. With RNA binding, however, it is not
generally sufficient to merely achieve hybridization;
the oligonucleotides must also usually participate in
the formation of a heteroduplex that is recognized by
30   RNase H. In the case of carbamate modifications,
hybridization with DNA and RNA does not occur,
possibly due to restricted rotation about the trigonal
carbamate linkage, thus eliminating their utility.
While capable of nucleic acid binding, neither
35   phosphoroamidates nor methyl phosphonates are able to

- 9 -

contribute to the formation of RNase H substrates.
Although, as discussed above, other mechanisms exist,
RNase H cleavage is the predominant route of post-
transcriptional control, limiting the usefulness of
5   these compounds as well. The polyamide-containing
oligonucleotide, on the other hand, has a
substantially increased affinity for nucleic acid,
relative to unmodified oligonucleotides. For example,
a polyamide chain containing ten thymidines (dT_{10}) when
10 mixed with a sequence of ten adenosines (dA_{10}) had a
melting temperature 50°C higher than an unmodified dT_{10}
oligonucleotide mixed with dA_{10}. While this could
yield useful therapeutic oligonucleotides, the
possibility exists that, along with the higher
15 affinity, could come a loss of sequence specificity.
     Oligonucleotides useful for therapeutic
applications should exhibit a high degree of
specificity and one preferably should be able to
produce such oligonucleotides with a high level of
20 selectivity. Any oligonucleotide containing phosphate
residues at which one of the peripheral phosphate
oxygens are modified, is chiral at the phosphorous.
Oligonucleotides, therefore, that contain such
modified phosphates are actually made up of a mixture
25 of diastereomers. Given that the number of
diastereomers is equal to 2^n, where n is the number of
chiral linkages in the oligonucleotide, such a mixture
can be very complex. For example, in an
oligonucleotide containing 15 modified residues, the
30 number of diastereomers in the mixture will be equal
to 2^{14}, or 16,384. Obviously, then, the presence of
modifications that yield chiral centers can severely
affect the oligonucleotides' specificity and the
ability to select a single oligomeric entity for
35 administration. Take the case of phosphorothioate, a

- 10 -

chiral modification, for example. Oligonucleotides
containing such a modification are only partially
nuclease resistant, with the level of nuclease
resistance being dependent upon both the specific
5   diastereomer being assayed and the specific nuclease
being used. Because individual diastereomers do not
react in a similar manner to each nuclease, it would
be extremely difficult to produce oligonucleotides
that are optimally nuclease resistant. Along with
10   phophorothioates, modifications including
phophoramidates and methyl phosphonates also yield
chiral centers. In addition to the problems involved
with chirality, lack of specificity may be exhibited
in other ways. Again using phosphorothioates as an
15   example, these compounds, due their hydrophobicity,
exhibit a whole range of non-specific effects,
including a general inhibition of transcription,
translation, DNA replication, and an inhibition of
kinase activity. Such effects can be dangerous, even
20   lethal. As stated above, the specificity of the
polyamide modified oligonucleotides is also
questionable.

        Before oligonucleotides can be used to inhibit
gene expression, the molecules must enter the cell.
25   Unmodified oligonucleotides are highly charged, having
roughly one full negative charge per nucleotide
residue, which generally results in a reduced rate of
transport across membranes, which can limit the
oligonucleotides' access to its ultimate site of
30   action. Nonetheless, unmodified oligonucleotides do
enter cells at a low, but finite rate (Heikkila et
al., 1987, Nature 328:445-449; Loke, S.L. et al.,
1989, Proc. Natl. Acad. Sci. USA 86:3474-3484).
Because passive diffusion of negatively charged
35   oligonucleotides across the plasma membrane is

- 11 -

limited, neutral analogs will enter cells more easily.
Of the oligonucleotide modifications described above,
most are neutral, with the phosphorothioate
modifications being the charged exception.

5   Finally, given the large scale on which these
oligonucleotide analogs must be produced, both for
therapeutic and diagnostic applications, the ease of
synthesis of the modified oligonucleotides must be
taken into account. Many of the modifications
10   mentioned above, such as the sulfur-containing
sulfide, sulfone and sulfoxide compounds, are hard to
synthesize. The methyl phosphonates are hard to make
and are also chemically unstable. Those modifications
that require difficult syntheses often offer only a
15   very small degree of flexibility as to their
composition. For example, the silyl compounds that
have been used as oligonucleotide modifications are
both acid and base labile, making their synthesis
difficult. Altering the structure of the silyl-
20   containing group in such a way that would make the
linkage more stable (e.g., by adding a t-butyl group)
is not possible because of an unacceptably high steric
hindrance that results due to the direct C-Si bond.
No silyl group, to date, has been reported that is
25   useful in oligonucleotide synthesis. Another example
of such a modification is the formacetal group. This
modification is achiral, neutral and yields
oligonucleotides that hybridize to nucleic acid. Its
synthesis, however, is prohibitively difficult.
30   Formacetal nucleoside monomer synthesis is very
complex, and no effective use of formacetal monomers
in the synthesis of oligonucleotides has been
reported, perhaps due to the fact that a radical
reaction is required for coupling to take place. Only
35   formacetal dimers have been used to add to growing

- 12 -

chains, limiting the possible composition of
formacetal-modified oligonucleotides. In fact, only
formacetal-containing pyrimidines have been reported,
further bringing into question the generality with
5   which this type of modification can be used.
Additionally, formacetal linkages, like the silyl-
containing ones described above, exhibit very limited
flexibility due to steric hindrance, further narrowing
the possible composition of oligonucleotides
10 containing formacetal linkages. Below is a table
summarizing the features of the modified
oligonucleotides that were described in this section:

	NUCLEASE RESISTANCE	NUCLEIC ACID HYBRIDIZATION	ACHIRALITY	NEUTRALITY	EASE OF SYNTHESIS FLEXIBILITY	SUBSTRATE FOR RNASE H
phosphorothioate[1]	+ /-	-	-	-	+	+ /-
phosphoramidate	+	+	-	+	+	-
methyl phosphonates	+	+	-	+	+	-
phosphate triesters	+	+	-	+	+ /-	-
carbamates	+	-	+	+	-	-
sulfide, sulfoxide, sulfone, sulfonyl	+	?	+	+	-	-
silyl	+	?	+	+	-	-
polyamide[2]	+	+ +	+	+	-	-
formacetal	+	+	+	+	-	-

30 _____

[1]   Due to their hydrophobicity, phosphorothioates
      exhibit a whole range of non-specific effects,
      including general inhibition of transcription,
      translation, DNA replication and an inhibition of
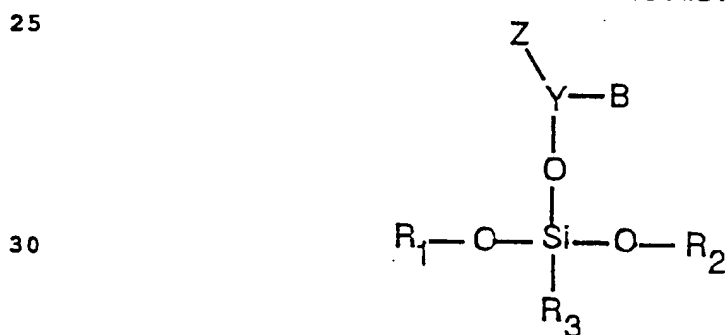   kinase activity.

35 [2]   The higher affinity exhibited for nucleic acid
      could lead to a loss of sequence specificity.

- 13 -

3.   SUMMARY OF THE INVENTION

The present invention presents a new class of
oligonucleotide analogs that contain one or more
5   stable internucleotide siloxy linkages. Such oligo-
nucleotide analogs may include deoxyribonucleotides or
ribonucleotides and may be single or double stranded.
This invention seeks to overcome the problems and
disadvantages of the prior art by providing
10   oligonucleotides that possess each of the features
required for the oligonucleotides to be used as
therapeutic drugs, modulators of specific gene
expression, or probes in diagnostic applications,
especially those applications in which significant
15   levels of nucleases may be present. These features
include ease of and flexibility in synthesis, achiral
centers, nuclease resistance, neutral charge, and
nucleic acid hybridization profiles that are
essentially equivalent to those of unmodified
20   oligonucleotides. None of the previously described
modifications can yield oligonucleotides that include
each of these properties.

The siloxy-containing monomers provided herein
are generally represented as follows:

25

$$
\begin{array}{c}
Z \\
\diagdown \\
Y\!-\!B \\
| \\
O \\
|
\end{array}
$$

30   $R_1\!-\!O\!-\!\underset{\underset{R_3}{|}}{\overset{\overset{O}{|}}{Si}}\!-\!O\!-\!R_2$

where Z is a protecting group, Y is a pentose sugar, B
is a nucleic acid base, R_1, and R_2, are apolar

35

- 14 -

moieties, and R_3 is a leaving group. In order for such
monomers to be achiral, R_1 must be the same as R_2.

The siloxy internucleotide linkages provided
herein are generally represented as follows:

$$
\begin{array}{c}
| \\
| \\
| \\
Y - B_1 \\
| \\
O \\
| \\
R_1 - O - Si - O - R_2 \\
| \\
O \\
| \\
Y - B_2 \\
| \\
|
\end{array}
$$
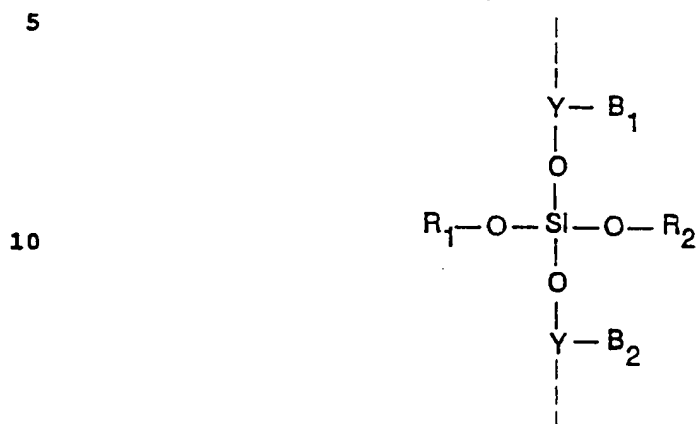
where R_1 and R_2 are apolar moieties, Y is a pentose
sugar, and B_1 and B_2 are nucleic acid bases. In order
for such linkages to be achiral, R_1 must be the same as
R_2. One or more of the phosphodiester linkages of the
oligonucleotides in this class may be substituted by
such siloxy linkages. Oligonucleotides useful as
therapeutic agents and other modulators of specific
gene expression range from about 10 to about 75
nucleotides in length, with about 15 to about 35
nucleotides being preferred. Nucleic acid molecules
useful as hybridization probes for diagnostic
applications range from about 15 to several thousand
nucleotides in length.

4.   BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Scheme for the preparation of 5'-3' d(T-
T) siloxy dimer phosphoramidite.

FIG 2: Photograph of gel showing siloxy links
are nuclease resistant. Lanes 1-3 contain unmodified

- 15 -

oligonucleotide 21-mers of the sequence depicted in
Section 6.4.1 below; lanes 4-6 contain siloxy
oligonucleotide analog 21-mers as were described in
Section 6.4.1 below. Lanes 1, 4: non-exonuclease-
5   treated controls; lanes 2, 5: partial exonuclease
digestions; lanes 3, 6: complete exonuclease
digestions.

5.   DETAILED DESCRIPTION OF THE INVENTION
10

A new class of oligonucleotide analogs that
contain one or more stable internucleotide siloxy
linkages is presented here. These linkages are
neutral, provide achiral centers around the silicon
15   atom, and yield oligonucleotides that possess each of
the features required for the oligonucleotides to be
used as therapeutic drugs, as other modulators of
specific gene expression, and as stable hybridization
probes for diagnostic applications. In addition to
20   achirality and a more neutral charge, these properties
include ease of and flexibility in synthesis, nuclease
resistance, and nucleic acid hybridization profiles
that are essentially equivalent to those of unmodified
oligonucleotides.
25   The composition of siloxy oligonucleotide analogs
and methods for the synthesis of such analogs is
presented below. In addition, the uses for such
siloxy oligonucleotide analogs is discussed. Examples
are presented of syntheses of siloxy monomers, dimers,
30   and oligonucleotides, and, in addition, it is
demonstrated that siloxy oligonucleotide analogs are
nuclease resistant and exhibit normal nucleic acid
hybridization profiles.


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- 16 -

5.1 SILOXY OLIGONUCLEOTIDE ANALOGS

The new class of oligonucleotide analogs described in this invention is the first to be
5 produced that contains one or more siloxy internucleotide linkages. In fact, until this point, the only utilization of siloxy groups with respect to oligonucleotide synthesis has been as reversible protecting groups. See for example, Markiewicz and
10 Adrych (Markiewicz, W.T. and Adrych, A., 1988, Nucleos. and Nucleot. 7:671-674) and Ogilvie and Pon (Ogilvie, K.K. and Pon, R.T., 1980, Nucl. Acids Res. 8:2105-2115) in which di-t-butoxy silyl groups are used as reversible protecting groups. Thus, it has
15 until now been assumed that the use of a siloxy group in the formation of a chemically stable internucleotide linkage, which is the central feature of this invention, would be unfeasible.

Not only, however, are these siloxy linkages
20 chemically stable. In addition, the siloxy linkages and the resulting siloxy containing oligonucleotides that contain such linkages are the first class of oligonucleotides described that possess each of the features necessary to make the therapeutic and
25 diagnostic use of oligonucleotides optimally efficacious. First, the linkages are uncharged, and therefore contribute to bringing oligonucleotide charge closer to neutral, thus increasing the oligonucleotides' cell permeation capabilities.
30 Second, siloxy linkages are nuclease resistant, which is a requirement due to the nuclease activity that is prevalent in serum and tissues which quickly degrades unmodified oligonucleotides, severely limiting their effectiveness. In addition, the silicon in siloxy
35 linkages is very similar, or isoteric, to the

- 17 -

phosphate in phosphodiester linkages, contributing to
excellent nucleic acid hybridization properties for
siloxy containing oligonucleotides, which are another
prerequisite for success when using oligonucleotides
5   to modulate gene expression as well as when using them
as hybridization probes in diagnostic applications.
Finally, siloxy linkages allow for a large degree of
selectivity and flexibility in their composition and
in the composition of oligonucleotides that contain
10 such linkages. Because achiral centers around the
silicon can be created, specific, pure oligonucleotide
compositions can be produced. The siloxy linkages are
chemically stable, are easy to synthesize, and, unlike
the direct C-Si bond present in silyl compounds
15 (Ogilvie, K.K. and Cormier, J.F., 1985, Tetrahedron
Lett. 26:4159-4162, for example), do not exhibit a
prohibitively large degree of steric hindrance, which
allows for a potentially large array of modifications
that can be incorporated into the siloxy group (see
20 description of R_1 and R_2 below). This flexibility
makes it possible, for example, to vary the
hydrophobicity of a given oligonucleotide such that
its rate of intracellular uptake is enhanced.

     The siloxy containing monomers provided herein
25 can be represented in (I), solely for purposes of
illustration and description and not by way of
limitation:

30

$$\begin{array}{c} Z \\ \backslash \\ Y-B \\ | \\ O \\ | \\ R_1-O-\underset{|}{Si}-O-R_2 \\ R_3 \end{array} \qquad (I)$$

35
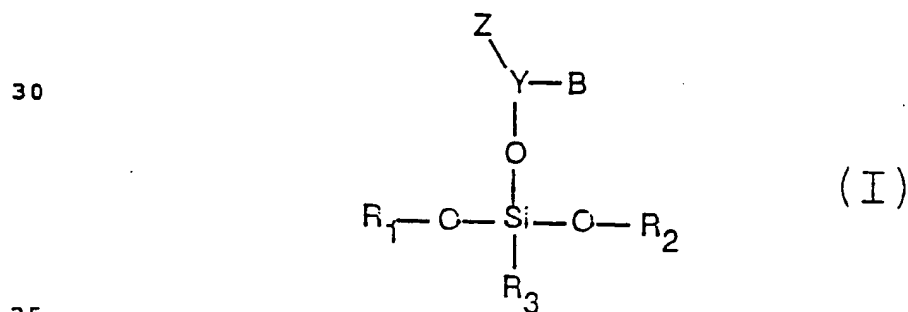
- 18 -

where Z is a protecting group, Y is a pentose sugar, B
is a nucleic acid base, R_1 and R_2 are apolar moieties,
and R_3 is a leaving group. The protecting group, Z,
can include, but is not limited to trityl,

5   monomethoxytrityl, dimethoxytrityl, pixyl,
phenoxyacetyl (PAC) or dimethylformamidine (DMF)
groups, with PAC and DMF groups being preferred. Y, a
pentose sugar, may consist of ribose, deoxyribose,
altered sugar configurations (e.g., arabinosides or

10 alpha-ribosides), or sugars with halogen
substitutions. The nucleic acid base, B, can include,
but is not limited to, the naturally occurring bases
(e.g., adenine, cytosine, guanine, thymine, or
uracil), and synthetically modified nucleic acid bases

15 such as inosine, bases with alkylated amino groups
(e.g., 1-methyladenosine, 7-methylguanosine, or $\underline{N}^6$-
isopentyladenosine), and azanucleic acid bases (e.g.,
deazaadenosine, 6-azauridine, 6-azathymidine). R_1 and
R_2 are apolar moieties that can include, but are not

20 limited to straight-chain or branched alkyl groups
(ranging in size from 1 to about 12 about carbon atoms
that can be attached to -O at any of their carbon
atoms), and aromatic groups. R_3, the leaving group,
can include, but is not limited to a halogen atom,

25 hydroxyl group, amine moiety, or acetate moiety. In
order for such monomers to be achiral, the R_1 moiety
must be the same as the R_2 moiety.

The siloxy internucleotide linkages provided
herein can be represented as in (II), solely for
30 purposes of illustration and description and not by
way of limitation:

35

- 19 -

$$Y - B_1$$
$$O$$
$$R_1 - O - Si - O - R_2 \qquad (II)$$
$$O$$
$$Y - B_2$$
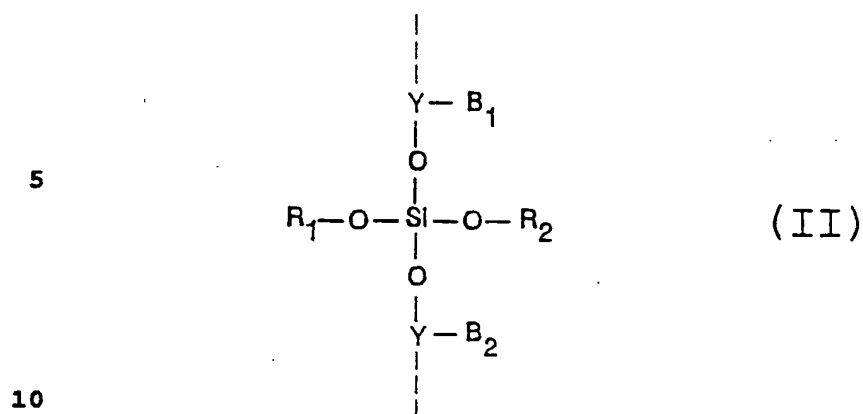
where R_1 and R_2 are apolar moieties as are described in
(I), Y is a pentose sugar as described in (I), and B_1
and B_2 are nucleic acid bases, as is described for B in
(I). One or more of the phosphodiester linkages in an
oligonucleotide are substituted by such siloxy
linkages.

Oligonucleotides useful as therapeutic agents and
as other modulators of specific gene expression range
from about 10 to about 75 nucleotides in length, with
about 15 to about 35 nucleotides being preferred.
Nucleic acid molecules useful as hybridization probes
for diagnostic applications range from about 15 to
several thousand nucleotides in length. Molecules up
to about 200 nucleotides may be synthesized using
standard methods, while molecules longer than this may
be obtained by ligating synthesized molecules together
and/or by ligating synthesized and naturally occurring
molecules together.

By way of further illustrating the siloxy
internucleotide linkages, one possible configuration
for such a linkage is presented in (III), solely for
purposes of illustration and description and not by
way of limitation:

- 20 -

(III)
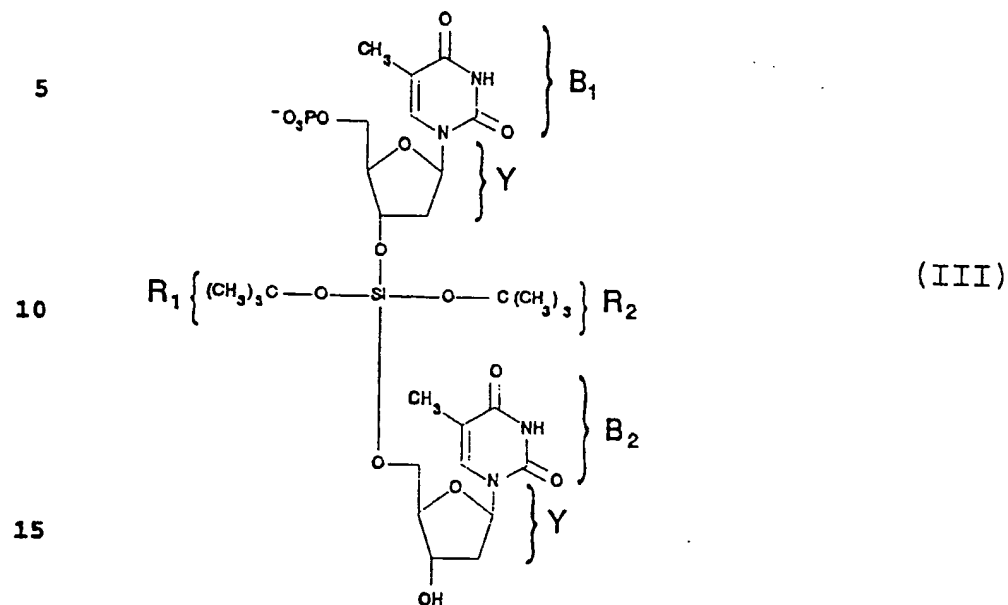
(III) represents an oligonucleotide consisting of 2
nucleotides connected by an achiral siloxy
internucleotide linkage, in which Y, the pentose
sugar, is deoxyribose, B_1 and B_2, the nucleic acid
bases, are both thymine, and R_1 and R_2, the apolar
moieties added to the siloxy group, are both t-butyl
alkyl groups. Syntheses of representative siloxy
monomers, dimers, and oligonucleotides are described
in the examples in Section 6.

The oligonucleotides of this invention may be
modified to best suit the particular purpose they are
to be used for. In the case of oligonucleotide
analogs to be utilized for antisense purposes, i.e.,
to modulate gene expression by binding to a target
mRNA, the siloxy oligonucleotides can be produced in a
number of ways. In order to take advantage of RNase H

- 21 -

cleavage, the oligonucleotide must be composed of
deoxyribonucleotides. In addition, to maximize RNase
H affinity for the heteroduplex formed between mRNA
and the oligonucleotide analog, not all of the

5 oligonucleotide's internucleotide linkages can be
siloxy linkages. The oligonucleotide should contain a
consecutive stretch of at least about four
nucleotides, and preferably at least about seven
nucleotides, connected by unmodified phosphodiester

10 bonds. Most preferably, all the remaining
internucleotide linkages flanking the stretch that is
to participate in RNase H substrate formation should
be siloxy linkages. If antisense oligonucleotides are
to be utilized to sterically, rather than

15 enzymatically, block translation, the oligonucleotides
may contain siloxy linkages at every internucleotide
linkage. Oligonucleotides to be used for antisense
purposes may range may about 10 to about 75
nucleotides, with about 15 to about 35 being

20 preferred.
       Oligonucleotides to be used in triplex helix
formation should be single stranded and composed of
deoxynucleotides. The oligonucleotides may contain
siloxy linkages at each of the internucleotide

25 positions. The base composition of these
oligonucleotides must be designed to promote triple
helix formation via Hoogsteen base pairing rules,
which generally require sizeable stretches of either
purines or pyrimidines to be present on one strand of

30 a duplex. Siloxy oligonucleotide sequences may be
pyrimidine-based, which will result in TAT and CGC+
triplets across the three associated strands of the
resulting triple helix. The pyrimidine-rich
oligonucleotides provide base complementarity to a

35 purine-rich region of a single strand of the duplex in

- 22 -

a parallel orientation to that strand. In addition,
oligonucleotides may be chosen that are purine-rich,
for example, contain a stretch of G residues. These
oligonucleotides will form a triple helix with a DNA

5   duplex that is rich in GC pairs, in which the majority
of the purine residues are located on a single strand
of the targeted duplex, resulting in GGC triplets
across the three strands in the triplex.
Alternatively, the potential sequences that can be

10 targeted for triple helix formation may be increased
by creating a so called "switchback" oligonucleotide.
Switchback oligonucleotides are synthesized in an
alternating 5'-3', 3'-5' manner, such that they base
pair with first one strand of a duplex and then the

15 other, eliminating the necessity for a sizeable
stretch of either purines or pyrimidines to be present
on one strand of a duplex.

The siloxy oligonucleotides to be used as
aptamers can be completely or partially modified. The

20 oligonucleotides may be composed of ribonucleotides or
deoxyribonucleotides, and may be either single or
double stranded.

The oligonucleotides to be used as ribozymes must
be composed of ribonucleotides, and the siloxy

25 linkages should, preferably, be located at only the 3'
and 5' terminal internucleotide positions. The
composition of the ribozyme oligonucleotides must
include one or more sequences complementary to a
target mRNA, and must include the catalytic sequence

30 responsible for mRNA cleavage. For this sequence, see
U.S. Pat # 5,093,246 (Been, M.D. et al., 1992) which
is incorporated by reference herein in its entirety.

35

- 23 -

5.2   SYNTHESIS OF SILOXY
      OLIGONUCLEOTIDE ANALOGS

      In all methods now commonly used,
5 oligonucleotides are synthesized from the 3' to the 5'
   end of the chain. These methods are discussed in
   "Oligonucleotide Synthesis: A Practical Approach"
   (Gait, M.J., ed., 1984, IRL Press, Oxford), which is
   incorporated in its entirety herein by reference.
10 With solid phase synthesis, the first residue is
   coupled to a solid support, such as polystyrene,
   silica gel, controlled pore glass beads,
   polyamide/Kieselguhr, or cellulose paper, through the
   3'-OH group. With solution phase synthesis, the 3'-OH
15 group of the first residue is blocked by a protecting
   group which is removed at the completion of the
   synthesis. Usually, one nucleoside unit, a nucleoside
   monomer, is added at a time to the 5'-OH group of the
   growing chain. Alternatively, rather than a monomer,
20 a block of two or more residues may be added in a
   single reaction step. Presented below is a discussion
   of schemes for monomer, dimer, and oligonucleotide
   synthesis of siloxy containing analogs.
   Representative examples of such syntheses are
25 presented in Section 6.
      To synthesize siloxy monomers, i.e., single
   siloxy nucleoside units, nucleosides with protected
   5'-OH groups (and 2'-OH groups, if
   oligoribonucleotides are being synthesized) may be
30 reacted, in the presence of appropriate catalysts,
   well known in the art, with silyl compounds including,
   but not limited to, silyl halides (e.g. silyl
   chloride), silylamines, silyl acetates, or silanols.
   Nucleosides may be, for example, reacted with silyl
35 halides in the presence of pyridine and

- 24 -

dichloromethane; reacted with silylamines in the
presence of pyridine, dichloromethane, and
dimethylamine; reacted with silyl acetates in the
presence of acetic acid, methanol, and hexanes;
5 reacted with silanols in the presence of
dichloromethane and pyridine. The silicon of the
silyl compounds may have alkyl groups, straight
chained or branched, of 1 to about 12 carbon atoms,
and/or aromatic groups, attached via siloxy linkages
10 (-O-Si-). Representative syntheses of siloxy monomers
are presented in the examples in Section 6.2.

Siloxy dimers may be synthesized using the siloxy
monomers described above, reacted with nucleosides
containing 3'-OH protecting groups. The halide,
15 amino, acetate, or hydroxyl groups, (e.g., R_3, in (I)
above) of the monomers react with the unprotected 5'-
OH group of the non-siloxy nucleoside to yield a
dimer. The 3' protecting groups are then removed
using standard techniques known in the art.
20 Representative examples of siloxy dimer syntheses are
presented in the examples in Sections 6.3, and
representative examples of 3' deprotection reactions
are presented in the examples in Section 6.5.

Siloxy-containing oligonucleotides may be
25 synthesized using the siloxy monomers and/or siloxy
dimers described above. First, a siloxy monomer may
be added, using the same techniques described above
for dimer synthesis, to the unprotected 5'-OH end of a
growing oligonucleotide chain. Second, a siloxy dimer
30 may be incorporated into a longer chain. The 3'
protecting group of the dimer is removed by standard
means, at which time a coupling group, such as a
phosphite-triester (e.g., phosphoramidite) or a
phospho-triester is added. The 5' protecting group of
35 the growing chain is removed and the chain is extended

- 25 -

by reacting it with the dimer, in the presence of a
catalyst and/or coupling agent. Both siloxy monomers
and dimers may be utilized in solution and solid phase
oligonucleotide syntheses, and may be used in manual
5 as well as automated, large scale oligonucleotide
syntheses. Double stranded oligonucleotides may be
produced by synthesizing complementary single stranded
oligonucleotides, using the techniques described
above, and then allowing these oligonucleotides to
10 anneal.

    Oligonucleotides to be used as hybridization
probes for diagnostic applications may be labeled with
radioactive, fluorescent, enzymatic, or chromogenic
moieties using standard procedures well known in the
15 art (Ausubel et al. eds., 1989, "Current Protocols in
Molecular Biology", Vol. 1, John Wiley Pub., New York;
Sambrook et al. eds., 1989, "Molecular Cloning", Cold
Spring Harbor Laboratory Press, Cold Spring Harbor).
Representative examples of siloxy oligonucleotide
20 syntheses are presented in Section 6.4, while
representative examples of 3' deprotection reactions
are presented in the examples in Section 6.5.

    5.3 USES AND ADMINISTRATION OF SILOXY
25   OLIGONUCLEOTIDE ANALOGS

    The siloxy oligonucleotide analogs of this
invention may be utilized for several purposes.
First, the siloxy oligonucleotides of the invention,
30 in the antisense, aptamer, triplex, or ribozyme
configurations described above, may be used as
therapeutic agents to block or reverse various
deleterious processes by modulating gene expression.
These include, but are not limited to, viral and
35 bacterial infection and/or replication, and any

- 26 -

inherited or acquired genetically induced disorders, including, but not limited to, those genetic lesions that cause malignant, or cancerous, growth to develop.

The modulation of gene expression necessary to
5 ameliorate such processes need not only be a down regulation. Oligonucleotides designed to enhance specific gene expression, in addition to those that are designed to inhibit specific gene expression, may both be useful as therapeutic agents. Oligonucleotide
10 enhancement of gene expression may be brought about, for example, by causing the repression of a negative regulatory transcription factor which, in turn, leads to enhancement of the target gene's expression.

For therapeutic uses, the siloxy oligonucleotides
15 of the invention may be formulated and administered through a variety of means, including systemic, and localized, or topical, administration. Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences," Mack Publishing
20 Co., Easton, PA, latest edition. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the oligonucleotides of the invention are formulated in aqueous solutions,
25 preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In addition, the oligonucleotides may be formulated in solid or lyophilized form, then redissolved or suspended
30 immediately prior to use. Systemic administration may also be accomplished by transmucosal, transdermal, or oral means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such
35 penetrants are generally known in the art.

- 27 -

Transmucosal administration may be through nasal
sprays or suppositories. For oral administration,
oligonucleotides may be formulated into capsules,
tablets, and tonics. For topical administration, the
5 oligonucleotides of the invention are formulated into
ointments, salves, gels, or creams, as is generally
known in the art.

Alternatively, the siloxy oligonucleotides of the
invention may first be encapsulated into liposomes,
10 then administered as described above. Liposomes are
spherical lipid bilayers with aqueous interiors. All
molecules that are present in an aqueous solution at
the time of liposome formation (in this case,
oligonucleotides) are incorporated into this aqueous
15 interior. The liposomal contents are both protected
from the external microenvironment and, because
liposomes fuse with cell membranes, are efficiently
delivered into the cell cytoplasm.

In addition to their therapeutic uses, the siloxy
20 oligonucleotide analogs, in antisense, aptamer,
triplex or ribozyme configurations, as described
above, may be used in any case where it is necessary
to modulate gene expression. These cases may include
industrial, agricultural, or research applications and
25 may involve cell culture systems in addition to
intact, multicellular organisms. As discussed above
for therapeutic applications, such siloxy
oligonucleotide modulation of gene expression may
involve either activation or repression of specific
30 gene activity.

The introduction of siloxy oligonucleotide
analogs into organisms and cells for such purposes may
be accomplished by several means. For mammalian
administration, each of the techniques described above
35 for therapeutic oligonucleotide purposes may be used.

- 28 -

In addition, other standard techniques for
introduction of nucleic acids into cells, including,
but not limited to, electroporation, microinjection,
and calcium phosphate precipitation techniques may be
5 utilized.

Alternatively, the siloxy oligonucleotides of the
invention may be used as diagnostic reagents to detect
the presence or absence of the target DNA or RNA
sequences to which they bind. Such diagnostic tests
10 may be conducted by hybridization through base pair
complementarity or triple helix formation which can
then be detected by conventional means. For example,
the oligonucleotides may be labeled using radioactive,
fluorescent, or chromogenic labels, all of which may
15 be detected using well known procedures. In addition,
the presence of a triple helix may be detected using
antibodies which specifically recognize these forms.
The diagnostic use of siloxy oligonucleotides is
especially advantageous when applied to procedures in
20 which the specimens to be analyzed may contain
significant levels of nucleases, as, for example, is
the case with in situ hybridizations.

        6.   EXAMPLE: SYNTHESIS OF SILOXY COMPOUNDS
25

        6.1 MATERIALS AND METHODS

        Nucleosides were obtained from Sigma Chemical Co.
(St. Louis, MO) and Peninsula Laboratories Inc.
30 (Belmont, CA). Silylating agents were from Aldrich
Chemical Co. (Milwaukee, WI). Dichloromethane and
pyridine were anhy· :ous and were purchased from
Aldrich. Acetonitrile was distilled from and stored
over calcium hydride (CaH$_2$). Deuterated solvents were
35 from Aldrich and stored over 4A molecular sieves.

- 29 -

Silica gel used for column chromatography was
Merk 60 from Aldrich and was used in the ratio of 10-
15 grams (g) of silica gel per gram of crude material.
Analytical thin layer chromatography (TLC) was done on
5 silica gel 60 F254 aluminum-backed plates purchased
from P.J. Cobert Associates (St. Louis).

^{1}H, ^{13}C and ^{29}Si NMR were obtained using Bruker WM-
360 MHz and AC-300 MHz spectrometers. All
measurements are relative to TMS (tetramethyl silane),
10 which was used as internal reference.

   6.2 SYNTHESIS OF SILOXY MONOMERS

   6.2.1   SYNTHESIS OF DI-T-BUTOXYDICHLOROSILANE
15   (Compound 1, FIG 1)

   (The compounds synthesized in sections 6.2.1 and
6.2.2 may be used in the synthesis of siloxy
oligonucleotide monomers. A demonstration of this is
20 presented below in section 6.2.3.)
   Di-t-butoxydichlorosilane was prepared according
to a literature procedure:
   G.W. Pedlow, Jr., and C.S. Miner, Jr., U.S.
Patents 2,556,363; 2,566,364; 2,566,365; 2,566,956 and
25 2,566,957, September 4, 1951.
   In a 1 liter round bottom flask equipped with an
addition funnel, reflux condenser, and mechanical
stirrer, was placed 86 milliliters (ml) (0.75 moles
(mol), 1.0 equivalent (equiv)) of silyl chloride
30 (SiCl$_4$) and 350 ml of dry toluene. This reaction
mixture was cooled to 0°C and 130 ml (1.6 mol, 2.1
equiv) of pyridine was added dropwise. A white
precipitate developed immediately and heat evolved.
The reaction was allowed to warm up to room
35 temperature and was stirred for 30 minutes. Then, 142

- 30 -

ml (1.5 mol, 2.0 equiv) of t-butanol was added all at
once and the reaction was stirred at room temperature
for 2 hours. The reaction mixture was then heated to
reflux for 1 hour. The reaction mixture was cooled,
5 an additional 300 ml of toluene was added and the
reaction mixture was filtered. The filtrate was
cooled to -20° C and refiltered if any additional
precipitate was seen. The solvent was removed and the
product was distilled under vacuum. A single product
10 was collected: boiling point (bp)= 72° C (20
millimeters (mm) Hg), Weight= 155 grams (g), Percent
yield= 85%. ^{29}Si NMR (CDCl$_3$): 72.2 parts per million
(ppm); ^{1}H NMR (CDCl$_3$) 1.42 ppm.

15   6.2.2   SYNTHESIS OF DI-T-BUTOXYDI-
                      METHYLAMINOSILANE

Reagents used:
1.   Dimethylamine
20 2.   Di-t-butoxydichlorosilane
3.   Benzene
Method: Dimethylamine (66.5 mmol, 30 g) was condensed
into a round bottom flask in a dry ice-acetone bath,
and was slowly transferred into 150 ml of a di-t-
25 butoxydichlorosilane (11.0 mmol, 26 ml)/benzene
solution at approximately -78°C. A white precipitate
was formed immediately. The reaction mixture was
warmed to room temperature over two hours. Excess
dimethylamine was released from the reaction vessel by
30 insertion of a needle with a drying tube on the top of
the rubber septum. The reaction mixture was stirred
overnight. The salt was then removed from the product
by filtration. Vacuum distillation afforded 47 g (83%
yield) of a colorless liquid, b.p. 93-94° C/21 mm Hg.
35

- 31 -

^{1}H NMR (CDCl$_3$) 1.8 (s, 12, N(CH$_3$)$_2$), 0.6 (s, 18, OC(CH$_3$)$_3$); ^{29}Si NMR (CDCl$_3$) - 67.2 ppm.

      6.2.3   SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-DI-T-BUTOXYCHLOROSILYLTHYMIDINE

Reagents used:
1. 5'-O-dimethoxytrityl-2'-deoxythymidine
2. Di-t-butoxydichlorosilane
3. Dichloromethane
4. Pyridine

Method: 5'-O-dimethoxytrityl-2'-deoxythymidine (1 mmol, 545 mg) was co-evaporated with anhydrous pyridine (3x10 ml) and was dissolved in 20 ml of dichloromethane. This solution was slowly transferred to a solution of di-t-butoxydichlorosilane (1 mmol, 0.237 ml) in 2 ml pyridine. The reaction mixture was stirred for three hours at room temperature under N$_2$ atmosphere, at which time silica gel TLC showed that the reaction was complete. ^{29}S: NMR (CDCl$_3$) - 82.7 ppm.

      6.2.4   SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-DI-T-BUTOXYHYDROXYSILYLTHYMIDINE

Reagents used:

1. 5'-O-dimethoxytrityl-di-t-butoxychlorosilylthymidine
2. Dichloromethane
3. Pyridine

Method: To a solution of 5'-O-dimethoxytrityl-di-t-butoxychlorosilylthymidine (5 mmol) in 20 ml dichloromethane, 5 ml pyridine was added 10 ml 10%

- 32 -

sodium carbonate (NaHCO$_3$) aqueous solution. The
reaction mixture was stirred for 30 minutes at room
temperature, was then extracted with water (3x20 ml),
the organic layer was separated, dried over sodium
sulfate (Na$_2$SO$_4$) for 30 minutes and was filtered.
Evaporation of the solvent gave a light yellow gum.
Flash silica gel column chromatography using a
gradient of hexanes:EtOAc, 1:0 to 7:3 with 1%
triethylamine gave 1.4 g of the product as a white
foam (37% yield). R$_f$ (EtOAc) 0.86; ^{1}H NMR (CDCl$_3$) 8.35
(bs, 1, N-H), 7.65 (s, 1, H6), 6.42 (m, 1, H1'), 4.48
(m, 1, H3'), 4.17 (m, 1, H4'), 3.76 (s, 6, OCH$_3$), 3.46-
3.36 (m, 2, H5'5"), 2.5-2.2 (m, 2, H2'2"), 1.4 (s, 3,
CH$_3$), 1.27 (s, 18, OC(CH$_3$)$_3$); ^{29}Si NMR (CDCl$_3$) - 91.10
ppm.

6.2.5   SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-DI-T-
BUTOXYDIMETHYLAMINOSILYLTHYMIDINE

Reagents used:
1. 5'-O-dimethoxytrityl-di-t-
butoxychlorosilylthymidine
2. Dichloromethane
3. Pyridine
4. Dimethylamine

Method: To a 20 ml solution of dichloromethane 6 ml
pyridine and 5'-O-dimethoxytrityl-di-t-
butoxychlorosilylthymidine (3.67 mmol) was added 10
equiv. of dimethylamine at -78°C. The reaction
mixture was slowly warmed to room temperature and was
stirred for 3 hours. The reaction mixture was
filtered, the white precipitate was washed with
dichloromethane and the filtrate was evaporated to
dryness. Purification was carried out by

- 33 -

chromatography on an Amberlite XAD-2 nonionic
polystyrene (50 g) column using a gradient of
Hexanes:Et$_3$N:EtOAc, 99:1:0 - 85:1:14 gradient. After
evaporation of the solvent, 2.04g (73% yield) of the
5 product as a white foam was obtained. ^{1}H NMR (CDCl$_3$)
6.5 (t, 1, H1'), 4.7 (m, 1, H3'), 4.2 (m, 1, H4'),
3.78 (s, 6, OCH$_3$), 3.5-3.3 (m, 2, H2'2"), 2.45 (s, 6
N(CH$_3$)$_2$), 2.5-2.2, (m, 2, H2'2"), 1.23 (s, 18,
OC(CH$_3$)$_3$); ^{29}Si NMR (CDCl$_3$) -79.4 ppm.
10

6.2.6   SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-DI-T-
          BUTOXYACETYLSILYLTHYMIDINE

Reagents used:
15 1.   5'-O-dimethoxytrityl-di-t-butoxychloro-
     silylthymidine
    2.   Dichloromethane
    3.   Triethylamine
    4.   Acetic anhydride
20

Method: Acetic anhydride (10.6 mmol, 1.0 ml) was
added to a solution of 5'-O-dimethoxytrityl-di-t-
butoxychlorosilylthymidine (0.41 mmol) in
dichloromethane (10 mL) and triethylamine (1 ml). The
25 reaction mixture was stirred at room temperature under
nitrogen overnight. Evaporation of the solvents gave
a yellow gum, which was purified by flash
chromatography on an alumina, basic column using a
gradient of CH$_2$Cl$_2$:Acetone, 10:0 to 5:5. ~~Rf(EtOAc)~~ J.W.
30 ~~0.92;~~ ^{29}Si NMR (CDCl$_3$) -97.3 ppm.   Z.L.

35

- 34 -

6.3   SYNTHESIS OF SILOXY DIMERS

6.3.1   SYNTHESIS OF 5'-O-THYMIDINE-DIMETHOXY-TRITYL-3'O-(5'-O-DI-T-BUTOXYSILYL)-3'-ACETYLTHYMIDINE

Reagents used:
1.   5'-O-dimethoxytrityl-di-t-butoxychlorosilylthymidine
2.   3'-O-acetyl-2'-deoxythymidine
3.   Dichloromethane
4.   Pyridine

Method: 3'-O-acetyl-2'-deoxythymidine (0.607 mmol, 172.6 mg) was added to a 20 ml dichloromethane and 2 ml pyridine solution of 5'-O-dimethoxytrityl-di-t-butoxychlorosilylthymidine (1.0 mmol) under nitrogen. The reaction mixture was stirred overnight at room temperature at which time silica gel TLC showed the reaction was complete.   The mixture was extracted with saturated $NaHCO_3$ aqueous solution (3x20 mL).   The organic layer was separated and dried over Na_2SO_4, and was filtered.   Evaporation of the solvent gave a yellow foam, which was dissolved in CH_2Cl_2 and was purified by flash chromatography on a silica gel column using solvent A(EtoAc:CH_2Cl_2:EtOH, 5:4.5:0.5):CH_2Cl_2, 0:1 to 2:3 as gradient.   5'-3' dimer was obtained as a white foam (440 mg, 73% yield based on 3'-O-acetyl-2' deoxythymidine added).   Rf(EtoAc) 0.57; ^{1}H NMR ($CDCl_3$), 9.6, 9.4 (bs, 2, NH), 7.6 (s, 1, H6), 7.5 (s, 1, H6), 6.4-6.3 (m, 2, H1'), 5.27 (d, 1, H2' from 3'-O-acetyl), 4.76 (d, 1, H3' from 5'-O-DMT), 4.1 (s, 1, H4' from 5'-O-DMT), 4.0 (s, 1, H4' from 3'-O-acetyl), 4.0-3.89 (m, 2, H5'5" from 3'-O-acetyl), 3.78 (s, 6, OCH_3) 3.5-3.32 (m, 2, H5'5" from 5'-ODMT),

- 35 -

2.47-2.27 (m, 4, H2'2"), 2.1 (s, 3, C(O)CH$_3$), 1.98 (s, 3, CH$_3$(T), from 3'-O-acetyl), 1.4 (s, 3, CH$_3$(T), from 5'-O-DMT, 1.28 (d, 18, OC(CH$_3$)$_3$); ^{29}Si NMR (CDCl$_3$) -91.1 ppm.

The 5'-O-dimethoxytrityl-thymidine-3'-O-di-t-butoxysilyl-5'-O-dimethoxytritylthymidine (3-3' dimer) was also isolated from the column as a white foam (76 mg, 10% yield). Rf(EtOAc) 0.70; ^{i}H NMR (CDCl$_3$) 9.0 (bs, 2, NH), 7.6 (s, 2, H6), 6.4 (q, 2, H1'), 4.7 (m, 2, H3'), 4.1 (d, 2, H4'), 3.76 (s, 12, OCH$_3$), 3.4-3.3 (m, 4, H5'5"), 2.4-2.2 (m, 4, H2'2"), 1.4 (s, 6, CH$_3$(T)), 1.27 (s, 18, OC(CH$_3$)$_3$); ^{29}Si NMR (CDCl$_3$) -92.2 ppm.

6.3.2   SYNTHESIS OF 5'-3' d(T-T) SILOXY DIMER PHOSPHORAMIDITE (COMPOUND 4, FIG. 1)

560 milligrams (mg) (1 millimole (mmol), 1.0 equiv) of 5' dimethoxy trityl (DMT) thymidine (T) was weighed in a flask, dried by coevaporation with pyridine, then dissolved in a mixture of 25 ml dry CH$_2$Cl$_2$ and 3 ml pyridine. To this mixture was added 0.24 ml (1 mmol, 1.0 equiv) of Cl$_2$Si(OtBu)$_2$ (compound 1, FIG. 1). The reaction mixture was then stirred under an inert atmosphere for 3 hours. At this time, silica gel thin layer chromatography (TLC) (EtOAc) indicated that all the starting material had reacted and two new spots were seen, corresponding to the desired monomer (compound 2, FIG. 1) and a small amount of 3'-3' T-T dimer. To this reaction mixture was then added 142 mg (0.5 mmol, 0.5 equiv) of 3' OAc T. The reaction mixture was then allowed to stir overnight, upon which time silica gel TLC of the reaction mixture indicated that all of the monomer had reacted and two spots were formed, corresponding to

5'-3' siloxy T-T dimer (R_f= 0.86) and 3'-3' siloxy T-T
dimer (R_f= 0.69). The two dimer species were separated
by careful flash chromatography on silica gel using a
stepwise gradient of CH_2Cl_2 plus 1.0% triethylamine
5 (TEA) to 1:1 CH_2Cl_2 plus 1.0% TEA/ 2:1 $Et_2O:CH_2Cl_2$. The
structure of the two compounds was verified by ^{29}Si and
^{1}H NMR. Yield of 5'3' dimer= 240 mg (47%). ^{29}Si NMR
($CDCl_3$): 91.1 ppm 5'-3' dimer, 92.1 ppm 3'-3' dimer.
    The OAc protecting group was removed from the 5'-
10 3' T-T dimer, as described below in Section 6.5.
    Finally, the phosphoramidite group was added to
the 3'-OH. Here, 95 mg (0.1 mmol, 1.0 equiv) 5'-3'
siloxy T-T dimer was placed in a flask and dissolved
in 4 ml dry CH_2Cl_2. To this solution, while stirring
15 under inert atmosphere, was added 40 microliters (μl)
(0.23 mmol, 2.3 equiv) of EtN(iPr)$_2$ diisopropyl
ethylamine and 45 μl (0.20 mmol, 2.0 equiv) of
ClP[N(iPr)$_2$](OCH$_2$CN). The reaction was then stirred
for 45 minutes, at which time silica gel TLC indicated
20 that the reaction was complete. The reaction was
quenched by the addition of a small amount of methanol
(CH$_3$OH) then diluted with the addition of 20 ml CH_2Cl_2.
The reaction mixture was washed with saturated NaHCO$_3$,
saturated sodium chloride (NaCl), dried over Na$_2$SO$_4$,
25 and then concentrated to give a yellow oil. The
reaction mixture was purified by flash chromatography
on silica gel using 30% Hexanes/EtOAc plus 1.0% TEA.
The fractions which contained the desired product were
then concentrated to give an off-white foam. Yield=
30 67.2 mg (58%). The foam was then dried under vacuum
and the structure was verified by NMR. R_f (EtOAc)
0.76. ^{1}H NMR (CDCl$_3$):7.65-6.28 m, 17H, ArH T H6 and
DMT; 6.44 t, 1H, H1'; 6.31 t, 1H, H1'; 4.76 m, 1H,
H3'; 4.56 m, 1H, H3'; 4.16 m, 1H, H4'; 4.09 m, 1H,
35 H4'; 3.95-3.32 m, 8H, H5', H5", and -NCH; 3.79 S. 6H,

DMT $-OCH_3$; 2.63 t, 2H, $-CH_2CN$; 2.48-2.04 m, 4H, H2',
H2"; 1.87 s, 3H, T $-CH_3$, 1.40 s, 3H T$-CH_3$; 1.28-1.04 m,
24H, $-OC(CH_3)_3$ and $-NC(CH_3)_2$. ^{29}Si NMR -87.4 ppm. ^{31}P
NMR -146.4 ppm.

6.4   SYNTHESIS OF SILOXY OLIGONUCLEOTIDES

6.4.1   EXAMPLE: SYNTHESIS OF SILOXY
                                        OLIGONUCLEOTIDE ANALOGS IN
                                        AUTOMATED DNA SYNTHESIS,
                                        USING 5'-3' d(T-T) SILOXY
                                        DIMER PHOSPHORAMIDITE

The 5'-3' siloxy T-T dimer prepared in the
example in Section 6.3.2 was used in the synthesis of
the following 21-mer:

5' T-T-C-A-G-G-C-T-C-TSiT-C-T-C-A-G-C-G-T-T-C   3'

where (-) represents a phosphodiester linkage and (Si)
represents a siloxy linkage.

Here, 125 mg of the siloxy dimer phosphoramidite
was weighed in a vial. This was dissolved in enough
anhydrous acetonitrile (CH_3CN) to give a 0.1 molar (M)
solution, filtered, then placed in the 5th base
position of an ABI (Applied Biosystems, Inc.) DNA
synthesizer. The normal 0.1 μmol synthesis cycle was
used except that the coupling time was extended to 5
minutes for the dimer. The trityl yield showed that
the coupling efficiency of the dimer addition was
about 90%. The oligonucleotide was then deblocked
using concentrated ammonium hydroxide (NH_4OH) at 55°C
for 7 hours. The product was purified from
untritylated sequences by reverse phase high
performance liquid chromatography (reverse phase
HPLC), detritylated, then re-purified by HPLC to

- 38 -

obtain the pure full length product. The length of
the product was then checked by electrophoresis on a
denaturing gel.

5   6.5   <u>DEPROTECTION OF 3'-O-ACETYL GROUP OF 5'-O-
              THYMIDINE-DIMETHOXYTRITYL-3'-O-(5'-O-DI-T-
              BUTOXYSILYL)-3'-ACETYLTHYMIDINE (COMPOUND 3,
              FIG. 1)</u>

10 <u>Reagents used</u>:
    1.   5'-O-dimethoxytrityl-thymidine-3'-O-(5'-O-di-
    butoxysilyl)-3'-acetylthymidine
    2. NH_4OH/EtOH, 3:1(v/v)

15 3.   EtOAc

    <u>Method</u>:   To 5'-O-dimethoxytrityl-thymidine-3'-O-(5'-O-
    di-t-butoxysilyl)-3'-acetylthymidine (0.26 mmol, 260
    mg) was added 36 ml of NH_4OH/EtOH (3:1(v/v)). The
20 solution was stirred overnight at room temperature.
    Silica gel TLC indicated that the removal of the
    acetyl was complete. Evaporation of the solvent gave
    a foam which was dissolved in 3 ml of EtOAc and was
    extracted with H_2O (2x2 ml). The organic layer was
25 collected and a white foam was obtained (206 mg, 83%
    yield). Rf(EtOAc) 0.30; ^1H NMR ($CDCl_3$), 7.6, 7.4 (s,
    2, H6), 6.4 (q, 1 H1' from 5'-O-DMT), 6.3 (q, 1, H1'
    from 3'-OH), 4.76 (d, 1, H3' from 5'-O-DMT), 4.48 (d,
    1, H3' from 3'-OH) 4.17 (s, 1, H4' from 3'-OH), 4.0
30 (m, 1, H4' from 5'-O-DMT), 3.96 (m, 2 H5'5" from 3'-
    OH), 3.79 (s, 6, OCH_3), 3.45-3.34 (m, 2, H5'5" from 5'-
    O-DMT), 2.44-2.02 (m, 4, H2'2"), 1.88 (s, 3, CH_3(T)
    from 3'-OH), 1.46 (s, 3, CH_3(T) from 5'-O-DMT), 1.3 (s,
    18, $OC(CH_3)_3$); ^{29}Si NMR ($CDCl_3$) -91.1. ppm.
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- 39 -

7.   EXAMPLE: <u>SILOXY INTERNUCLEOTIDE LINKAGES ARE</u>
                 <u>NUCLEASE RESISTANT</u>

        The siloxy oligonucleotide 21-mer described in
5   Section 6.4.1, above, and unmodified oligonucleotides
    of the same sequence, were used in this study to
    demonstrate that siloxy internucleotide linkages are
    nuclease resistant. For visualization purposes, the
    gels were stained with Stains-All (Sigma).
10   Unmodified and siloxy-containing 21-mers were
    reacted separately with calf spleen phosphodiesterase,
    a 5'-3' exonuclease. The exonuclease digestion
    reaction conditions were as follows: oligonucleotides
    were digested in 10 μl containing 100 mM sodium
15 succinate pH 6.1, 100 μM EDTA, and 0.2 μg/μl enzyme
    (Boehringer Mannheim). Partial digests were obtained
    by digesting the oligonucleotides for 8 minutes at
    37°C. More complete digests were obtained by
    increasing the reaction times to 30 minutes. Samples
20 were analyzed by electrophoresis on a 20%
    polyacrylamide/7M urea gel, run for 3 hours at 400
    volts. Gels were then stained with Stains-All.
        Figure 2 shows the results of one such set of
    oligonucleotide digests. Lanes 1-3 contain the
25 unmodified oligonucleotide 21-mer, lanes 4-6 are the
    corresponding lanes containing the siloxy
    oligonucleotide 21-mer analog. Lanes 1 and 4 are non-
    exonuclease treated controls showing the intact 21-
    mers. Lanes 2 and 5 represent partial exonuclease
30 digests which produce a ladder of bands. Lanes 3 and
    6 represent more complete exonuclease digests. From
    lanes 5 and 6 it is apparent that degradation stops at
    the siloxy linkage at position 10 of the 21-mer
    oligonucleotide analog, demonstrating that the siloxy
35 linkage is completely nuclease resistant.

- 40 -

8.   EXAMPLE: SILOXY OLIGONUCLEOTIDE ANALOGS AND
                  UNMODIFIED OLIGONUCLOTIES EXHIBIT
                  SIMILAR NUCLEIC ACID HYBRIDIZATION
                  PROFILES

5

The siloxy oligonucleotide 21-mer described in
Section 6.4.1, and unmodified oligonucleotides (DNA
and RNA) of the same and complementary sequence were
used in this study to show that siloxy oligonucleotide
10   analogs and unmodified oligonucleotides exhibit
similar nucleic acid hybridization properties.

Unmodified DNA and RNA oligonucleotides were
synthesized by the phosphoramidite method with
commercially available monomers. The 2'-OH of the RNA
15   monomers (Peninsula Laboratories) were protected with
the t-butyldimethylsilyl group. The T_m of the siloxy
21-mer and the corresponding unmodified
oligonucleotide, hybridized to either complementary
DNA or RNA sequences, was determined. Melting curves
20   were obtained under the following conditions: A total
oligonucleotide concentration of approximately 1 μM in
150 mM Nacl and 10 mM sodium phosphate buffer, pH 7.3.

The data obtained are summarized in the following
table:

25

	cDNA T_m	cRNA T_m
Siloxy 21-Mer	68.0°C	69.0°C
Unmodified 21-mer	70.0°C	70.5°C

These data show that the siloxy linkage has
30   little effect on the stability of a duplex with either
a complementary DNA (B-type helix) or RNA (A-type
helix) oligonucleotide. This is consistent with
molecular modeling studies of A- and B- type helices
which show that the t-butyl groups are on the exterior

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- 41 -

of the helix in both cases and can be well
accommodated without causing steric hindrance.

The siloxy linkage of the 21-mer is at position
10, the middle of the oligonucleotide, which was the
5 position that would have been most likely to affect
the oligonucleotide's hybridization properties.
Because even a siloxy linkage at this position has
essentially no effect on the oligonucleotide's
hybridization profile, it can be concluded that a
10 siloxy linkage can be placed at any position of the
oligonucleotide without adversely affecting its
hybridization properties.

15   It is apparent that many modifications and
variations of this invention as hereinabove set forth
may be made without departing from the spirit and
scope thereof. The specific embodiments described
below are given by way of example only and the
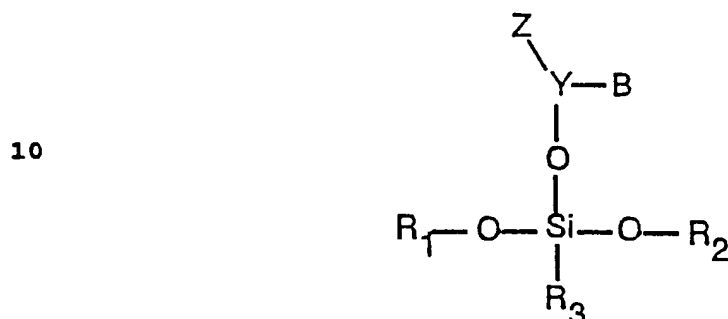20 invention is limited only by the terms of the appended
claims.

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- 42 -

CLAIMS

What is claimed is:

5   1.   A siloxy nucleotide analog having the
        formula

$$
\begin{array}{c}
Z \\
\diagdown \\
Y - B \\
| \\
O \\
| \\
R_1 - O - Si - O - R_2 \\
| \\
R_3
\end{array}
$$

15   where Z is a protecting group, Y is a
                pentose sugar, B is a nucleic acid base, R_1
                and R_2 are apolar moieties, and R_3 is a
                leaving group.

20   2.   The siloxy nucleotide analog of claim 1

                where Z is a protecting group selected from
                the group consisting of trityl,
                monomethoxytrityl, dimethoxytrityl, pixyl,
25   phenoxyacetyl, and dimethylformamide
                moieties;

                where Y is a pentose sugar selected from the
                group consisting of ribose moieties,
30   deoxyribose moieties, arabinoside moieties,
                and alpha-riboside moieties;

                where B is a nucleic acid base selected from
                the group consisting of naturally occurring
35

- 43 -

nucleic acid bases and synthetically
modified nucleic acid bases;

5   where R_1 and R_2 are apolar moieties selected
         from group consisting of aromatic moieties,
         and straight-chained alkyl moieties and
         branched-chain alkyl moieties, which alkyl
         moieties contain from 1 to about 12 carbon
         atoms and which alkyl moieties may be
10   attached to its adjoining oxygen atom, as
         depicted in the formula of claim 1, at any
         of such carbon atoms;

         where R_3 is a leaving group selected from the
15   group consisting of halogen moieties,
         alcohol moieties, amino moieties, and
         acetate moieties.

    3.   The siloxy nucleotide analog of claim 2
20
         where B is a nucleic acid base selected from
         the group consisting of adenine, cytosine,
         guanine, thymine, uracil, inosine, 1-
         methyladenosine, 7-methylguanosine, $\underline{N}^6$-
25   isopentyladenosine, deazaadenosine, 6-
         azaurdine, and 6-azathymidine.

    4.   The siloxy nucleotide analog of claims 1, 2,
    or 3 in which R_1 and R_2 are the same moieties.
30
    5.   The siloxy nucleotide analog of claim 4 in
    which R_1 and R_2 are t-butyl groups.

    6.   The siloxy nucleotide analog of claim 2
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- 44 -

where Z is a dimethoxytrityl group;

where Y is a pentose sugar selected from the group consisting of deoxyribose and ribose;

where B is a nucleic acid base selected from the group consisting of adenine, cytosine, guanine, thymine, and uracil;

where R_1 and R_2 are t-butyl groups; and

where R_3 is chlorine.

7.   The siloxy nucleotide analog of claim 2

where Z is a dimethoxytrityl group;

where Y is a pentose sugar selected from the group consisting of deoxyribose and ribose;

where B is a nucleic acid base selected from the group consisting of adenine, cytosine, guanine, thymine, and uracil;

where R_1 and R_2 are t-butyl groups; and

where R_3 is -OH.

8.   The siloxy nucleotide analog of claim 2

where Z is a dimethoxytrityl group;

where Y is a pentose sugar selected from the group consisting of deoxyribose and ribose;

- 45 -

where B is a nucleic acid base selected from
the group consisting of adenine, cytosine,
guanine, thymine, and uracil;

5   where R_1 and R_2 are t-butyl groups; and

where R_3 is dimethylamine.

9.   The siloxy nucleotide analog of claim 2
10
where Z is a dimethoxytrityl group;

where Y is a pentose sugar selected from the
group consisting of deoxyribose and ribose;
15
where B is a nucleic acid base selected from
the group consisting of adenine, cytosine,
guanine, thymine, and uracil;

20   where R_1 and R_2 are t-butyl groups; and

where R_3 is acetate.

25   10.   A nucleic acid analog comprised of two or
more nucleotides in which one or more of the
phosphodiester internucleotide linkages are
substituted by a siloxy linkage, said linkage having
the formula
30

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- 46 -

$$-W_1-O-\underset{\underset{\underset{R_2}{|}}{\overset{\overset{R_1}{|}}{\overset{O}{|}}}{\overset{}{Si}}-O-W_2-$$
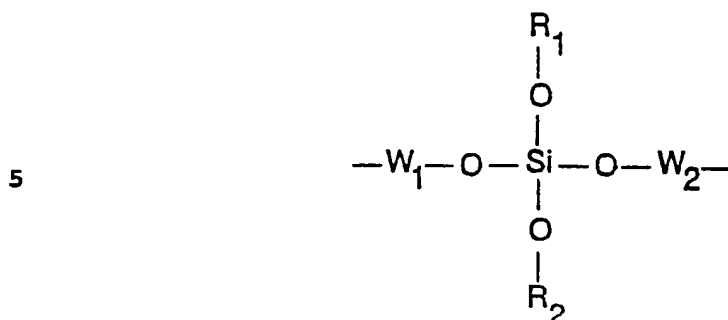
where R_1 and R_2 are apolar moieties selected from the group consisting of aromatic moieties, straight-chained alkyl moieties and branched-chain alkyl moieties, which alkyl moieties contain from 1 to about 12 carbon atoms and which alkyl moieties may be attached to its adjoining oxygen atom, as depicted in the formula, at any of such carbon atoms; and

where W_1 and W_2 are adjacent nucleotides in the nucleic acid that are linked via the siloxy internucleotide linkage.

11. The nucleic acid analog of claim 10 which is single stranded.

12. The nucleic acid analog of claim 10 which is double stranded.

13. The nucleic acid analog of claim 10 in which the R_1 and R_2 moieties within each individual siloxy internucleotide linkage are the same moiety.

14. The nucleic acid analog of claim 13 in which the R_1 and R_2 moieties within each siloxy internucleotide linkage are t-butyl groups.

- 47 -

15.   The nucleic acid analog of claim 10, 13, or
14 which ranges in length from about 10 to about 75
nucleotides.

5   16.   The nucleic acid analog of claim 10, 13, or
14 which ranges in length from about 15 to about 35
nucleotides.

17.   The nucleic acid analog of claim 15 in which
10   at least 4 consecutive nucleotides are connected by
unmodified phosphodiester internucleotide linkages.

18.   The nucleic acid analog of claim 15 in which
at least 7 consecutive nucleotides are connected by
15   phosphodiester internucleotide linkages.

19.   The nucleic acid analog of claim 17 in which
only the 3' and 5' terminal internucleotide linkages
are siloxy linkages.
20

20.   The nucleic acid analog of claim 18 in which
only the 3' and 5' terminal internucleotide linkages
are siloxy linkages.

25   21.   The nucleic acid analog of claim 17 in which
all internucleotide linkages outside said consecutive
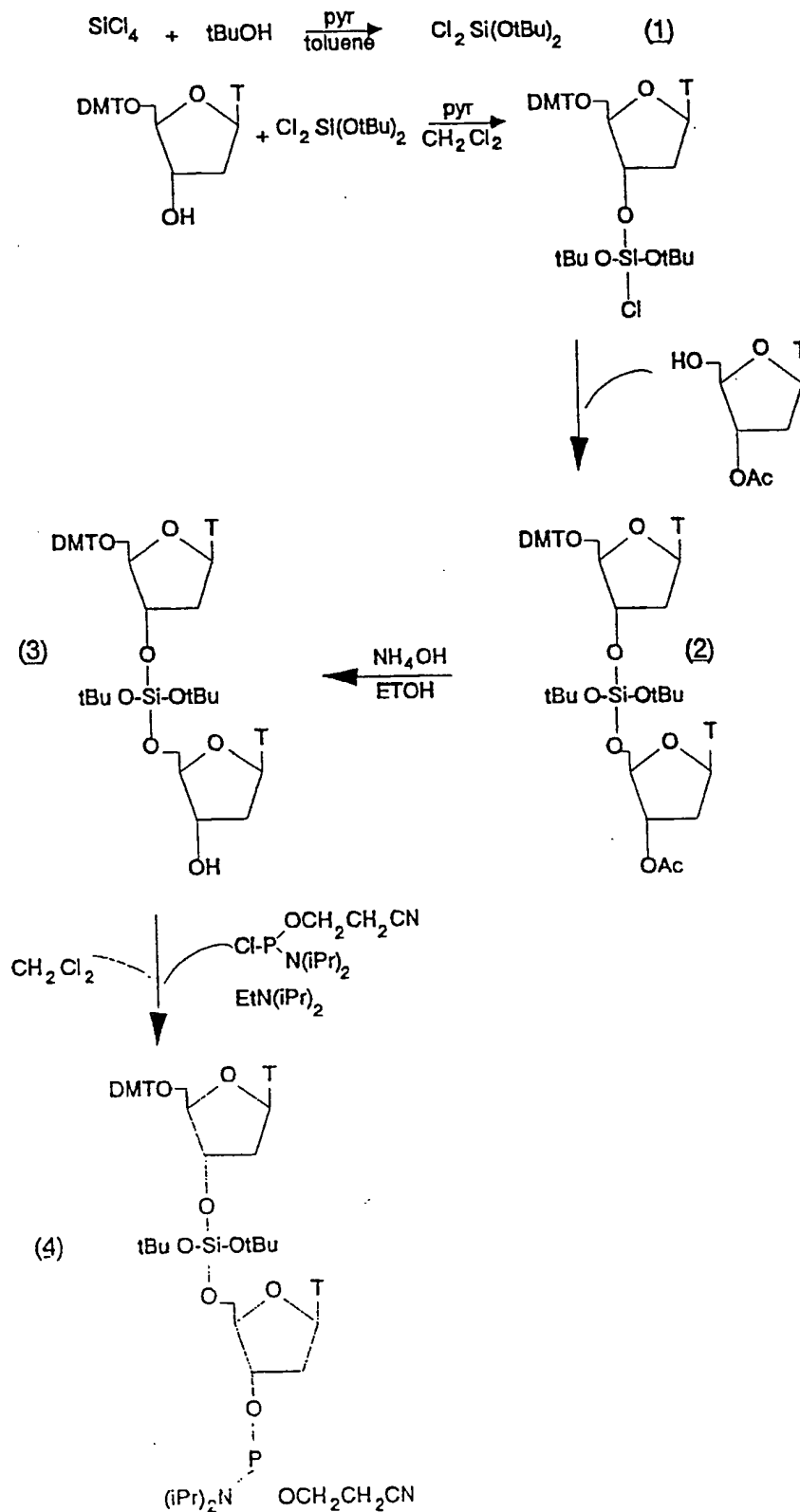stretch of phosphodiester internucleotide linkages are
siloxy linkages.

30   22.   The nucleic acid analog of claim 18 in which
all internucleotide linkages outside said consecutive
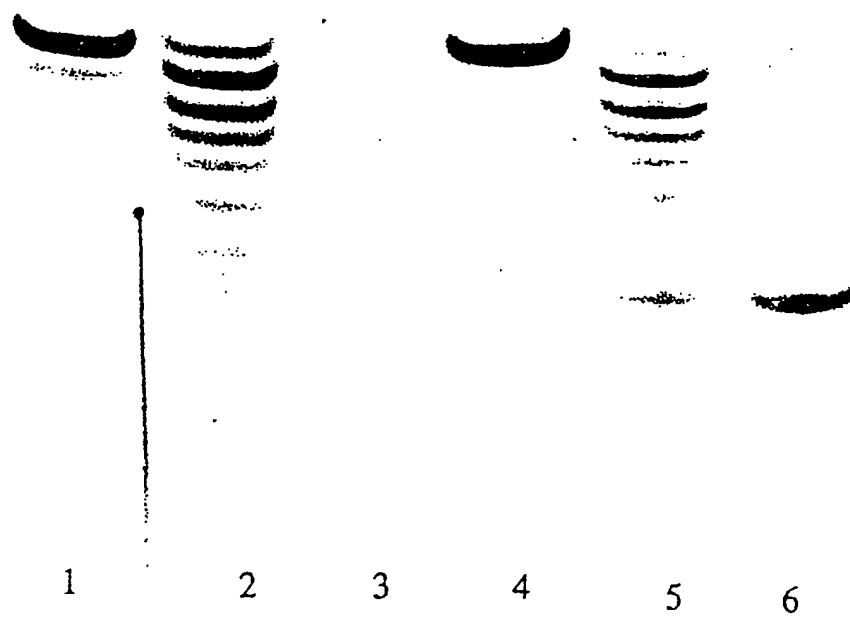stretch of phoshphodiester internucleotide linkages
are siloxy linkages.

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Figure 1

Scheme for the preparation of the 5'-3' d(T-T) siloxy dimer phosphoramidite

FIGURE 2

1   2   3   4   5   6

International application No.

PCT/US93/08980

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07H 19/00, 21/00, 17/00
US CL :536/22, 24.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/22, 24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUCLEIC ACIDS RESEARCH, Volume 16, No. 10, issued 1988, Cormier et al, "Synthesis of hexanucleotide analogues containing diisopropylsilyl internucleotide linkages", pages 4583-4594, see entire entire document.	1-22
A	TETRAHEDRON LETTERS, Volume 26, No. 35, issued 1985, Ogilvie et al, "Synthesis of a thymidine dinucleotide analogue containing an internucleotide silyl linkage", pages 4159-4162, see entire document.	1-22

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the 1564X international search report
01 November 1993	19 NOV 1993
Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ELIZABETH C. KEMMERER
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUCLEOSIDES AND NUCLEOTIDES, Volume 6, issued 1987, Seliger et al, "Oligonucleotide analogues with dialkyl silyl internucleoside linkages", pages 483-484, see entire document.	1-22
A	CHEMICAL REVIEWS, Volume 90, No. 4, issued June 1990, Uhlmann et al, "Antisense oligonucleotides: a new therapeutic principle", pages 543-584, see entire document.	1-22